A New Micro-fluidic Device for Protein Separation Fabricated on a Silicon Substrate

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The sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) has been widely used for protein separation because it is a simple and reliable method. With the recent introduction of micro-fabrication process into the micro-fluidic device field, successful separations of proteins using the micro-channel-based structure have been reported. However, the system often requires complicated fluorescence chemicals to identify proteins and a high operation electrical field.

A new micro-channel device fabricated on a silicon wafer has been developed. The channel structure was with the silicon technology including the lithography process, thin film deposition, and etching. The device has been effective in separating proteins from a mixture. In principle, it can also be used to further separate other biomolecules such as DNA.

Figure 1 shows the schematic view of the micro-channel device fabrication. A thin silicon nitride (first SiN_x) was deposited by plasma-enhanced chemical vapor deposition (PECVD) at 250°C and etched with the buffered oxide etchant BOE using the first mask. The first SiN_x was used as the hard mask for the definition of the main structure of the device that contains the micro-channel and two reservoirs. The silicon wafer was etched with the 30% KOH solution at 70°C. The influence of the addition of isopropyl alcohol (IPA) on the channel morphology was studied. The second PECVD SiN_x was deposited to cover the exposed silicon surface, which is followed by the deposition of the chromium film for the both load and detection electrodes using a RF magnetron sputtering system. The two electrodes were etched with a solution ($HCl:H_2O$ 3:1) using the second mask. A cellulose tape was attached to complete the channel structure.

For protein separation experiments, the channel was filled with the polyacrylamide gel that was subsequently polymerized. Three model proteins, i.e., ovalbumin (45kDa), carbonic anhydrase (29kDa) and α-lactalbumin (14.2kDa), were prepared by conventional methods of SDS-PAGE. Protein solutions with various concentrations were examined. The electrophoresis was carried out at 10 V/cm electric filed. The separation of different proteins was detected by measuring various peak times in the drastic current drop rate vs. time curve.

Figure 2 shows that three proteins in a mixture can be separated within 15 min using this kind of device with a short length channel of 500 μm. Arrival times of these proteins are clearly shown as the three peak times. Solutions containing one or two proteins were also tested. The peak time of each is not influenced by the existence of other proteins. Proteins on the detecting electrode were identified by the staining method using Coomassie brilliant Blue and the electron spectroscopy for chemical analysis (ESCA).

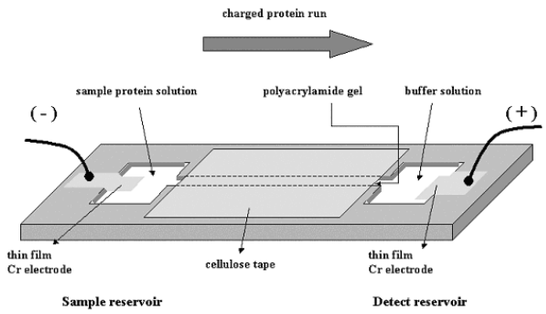

Figure 1 Schematic diagram of micro-channel fabrication

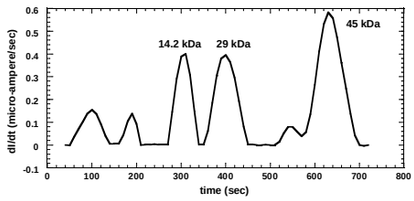

Figure 2 Current change rate vs. time of a mixture containing three different proteins

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